Remarks

Claims 1-19 and 27-63 are pending. Claims , 2, 13, 29 and 42 have been amended. Claims 20-26 have been canceled. Claims 60-63 are newly added. Claims 1, 2, 13, 29 and 42 were amended to more clearly claim what applicants consider to be their invention. Claims 1 and 42 have been amended to remove reference to elimination of unhybridized probes as an effect of treatment of the probes. All of the claims now require that the structured probes be treated to alter unhybridized probes. Only probes with intact duplex regions are altered. Claims 2, 13 and 29 have been amended to recite "duplex region" rather than "stem" to be consistent with the antecedent term "duplex region" recited in claim 1 to which claims 2, 13 and 29 refer.

New claim 60 depends from claim 1 and recites that the treatment is extending the second end of the probe using probe sequence as template. Support for new claim 60 can be found at least in original claim 2 and in Figures 1, 2d and 2e where extension of the second end of probes using probe sequence as template is depicted. New claim 61 depends from claim 60 and recites that the duplex region is a curl stem and loop structure. Support for new claim 61 can be found at least in original claim 34.

New claims 62 and 63 are based on claim 1, in which they find support. New claim 62 recites that the treatment is extending the second end of the probe using probe sequence as template. Support for new claim 62 can also be found at least in original claim 2 and in Figures 1, 2d and 2e where extension of the second end of probes using probe sequence as template is depicted.

Rejections Under 35 U.S.C. § 102

1. Claims 1-3, 8, 9 and 59 were rejected under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 6,277,607 to Tyagi et al. ("Tyagi I"). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

Tyagi I discloses a method of amplifying and detecting nucleic acid sequences using primers that form stem and loop structures. Tyagi I does not disclose altering any of the primers (or any probe) having intact stems and does not disclose altering only the primers having intact stems.

Claims 1-3, 8, 9 and 59 are drawn to, in part, a method of detecting target nucleic acid sequences comprising

(a) bringing into contact one or more target samples and one or more structured probes, and incubating under conditions that promote hybridization between target nucleic acid sequences in the target samples and the structured probes,

wherein each structured probe has a first end and a second end, wherein each structured probe comprises at least two complementary portions, a target probe portion, and a detection portion, wherein the two or more complementary portions comprise a first complementary portion and a second complementary portion,

wherein two or more of the complementary portions form a duplex region, wherein formation of the duplex region forms a loop, wherein at least a portion of the target probe portion is in the loop, wherein hybridization of the target probe portion to the target nucleic acid sequence disrupts the duplex region,

(b) detecting the structured probes, wherein, prior to detection, the structured probes are treated to alter unhybridized probes, wherein only probes with intact duplex regions are altered by treatment of the structured probes, wherein altered probes are not detected.

Significantly, claim 1 (from which the other claims depend) requires that two or more of the complementary regions of the structured probes form a duplex region together. Claim 1 also requires that this duplex region be disrupted when the structured probe hybridizes to the target nucleic acid sequence. Claim 1 also requires that only probes with intact duplex regions are altered. That is, only structured probes that do not hybridize to the target nucleic acid sequence are altered.

Tyagi I fails to disclose any alteration of the stem and loop primers used in the Tyagi I method. Tyagi I fails to disclose alteration of only those stem and loop primers with intact stems. Rather, the method of Tyagi I is based on priming of nucleic acid synthesis by those primers that hybridize to a target nucleic acid sequence and non-priming by those primers that do not (such primers cannot prime nucleic acid synthesis <u>because</u> they are not hybridized to a target). There is no alteration of the Tyagi I primers. Thus, Tyagi I fails to disclose at least these two features of claims 1-3, 8, 9 and 59: alteration of a structured probe and alteration of only

those structured probes with intact duplex regions. Accordingly, Tyagi I cannot anticipate claims 1-3, 8, 9 and 59.

Regarding claims 2, 8 and 9, Applicants note that claim 2 (from which the other claims depend) requires that the treatment of the structured probe is extension of the second end of the probe using probe sequence as template and that only probes with intact duplex regions are extended. Tyagi I fails to disclose either of these features. Tyagi I discloses extension (during amplification) of primers that are hybridized to a target nucleic acid sequence. Such primers are first of all not extended using probe sequence as template (rather, it is the target nucleic acid that serves as template). Such extension is also not limited to primers with intact stems (i.e. primers that did not hybridize to a target nucleic acid sequence). On the contrary, it is only those primers where the stem has been disrupted (i.e. those primers that hybridize to target) that are extended. Thus, on this point, Tyagi I discloses the exact opposite of the claimed method. Because Tyagi I fails to disclose extension of primers using primer sequence as template and fails to disclose extension of only primers with intact duplex regions, Tyagi I fails to anticipate claims 2, 8 and 9 for at least these additional reasons.

Regarding claim 3, Applicants note that claim 3 requires that the extended probe not be complementary to the primer complement of an amplification target circle. Because Tyagi I fails to disclose the extension required in claim 2 (from which claim 3 depends), Tyagi I does not disclose any property of such a probe or the probe required by claim 3. Because Tyagi I fails to disclose an amplification target circle, Tyagi I does not disclose any non-complementarity to any amplification target circle. For at least these additional reasons, Tyagi I fails to anticipate claim 3.

For all of the above reasons, claims 1-3, 8, 9 and 59 are not anticipated by Tyagi I.

2. Claims 1-3, 5-9, 20-28, 30-34 and 59 were rejected under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 5,925,517 to Tyagi et al. ("Tyagi II"). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

Tyagi II discloses a method of detecting nucleic acid sequences using probes that form stem and loop structures and that produce a detectable signal when the stem is disrupted. Tyagi

II does not disclose altering any of the probes having intact stems and does not disclose altering only the probes having intact stems.

Claims 1-3, 5-9, 27, 28, 30-34 and 59 are drawn to, in part, a method of detecting target nucleic acid sequences comprising

(a) bringing into contact one or more target samples and one or more structured probes, and incubating under conditions that promote hybridization between target nucleic acid sequences in the target samples and the structured probes,

wherein each structured probe has a first end and a second end, wherein each structured probe comprises at least two complementary portions, a target probe portion, and a detection portion, wherein the two or more complementary portions comprise a first complementary portion and a second complementary portion,

wherein two or more of the complementary portions form a duplex region, wherein formation of the duplex region forms a loop, wherein at least a portion of the target probe portion is in the loop, wherein hybridization of the target probe portion to the target nucleic acid sequence disrupts the duplex region,

(b) detecting the structured probes, wherein, prior to detection, the structured probes are treated to alter unhybridized probes, wherein only probes with intact duplex regions are altered by treatment of the structured probes, wherein altered probes are not detected.

Significantly, claim 1 (from which the other claims depend) requires that two or more of the complementary regions of the structured probes form a duplex region together. Claim 1 also requires that this duplex region be disrupted when the structured probe hybridizes to the target nucleic acid sequence. Claim 1 also requires that only probes with intact duplex regions are altered. That is, only structured probes that do not hybridize to the target nucleic acid sequence are altered.

Tyagi II fails to disclose any alteration of the stem and loop probes used in the Tyagi II method. Tyagi II fails to disclose alteration of only those stem and loop probes with intact stems. Rather, the detection method of Tyagi II is based only on hybridization versus non-hybridization of probes to a target nucleic acid sequence. There is no alteration of non-hybridized probes either disclosed or required in the method of Tyagi II because hybridization

alone determines signal generation in Tyagi II. Thus, Tyagi II fails to disclose at least these two features of claims 1-3, 5-9, 27, 28, 30-34 and 59: alteration of a structured probe and alteration of only those structured probes with intact duplex regions. Accordingly, Tyagi II cannot anticipate claims 1-3, 5-9, 27, 28, 30-34 and 59.

Regarding claims 2, 8 and 9, Applicants note that claim 2 (from which the other claims depend) requires that the treatment of the structured probe is extension of the second end of the probe using probe sequence as template and that only probes with intact duplex regions are extended. Tyagi II fails to disclose either of these features. Tyagi II does not disclose extension of probes. Rather, Tyagi II discloses (in the passage cited in the Office Action) universal stems (which can have a single-stranded overhang) that can be joined to target sequences to produce a probe for use in the method of Tygai II (col. 19, lines 5-14). Such universal stems are not structured probes and are not extended using probe sequence as template. The amplification (col. 20, lines 40-56) cited in the Office Action refers to general amplification of sample nucleic acids in preparation for probe hybridization. Nothing in the cited passage indicates that the probes are extended in the amplification reactions. Because Tyagi II fails to disclose extension of structured probes using probe sequence as template, Tyagi II fails to anticipate claims 2, 8 and 9 for at least these additional reasons.

Regarding claim 3, Applicants note that claim 3 requires that the extended probe not be complementary to the primer complement of an amplification target circle. Because Tyagi II fails to disclose the extension required in claim 2 (from which claim 3 depends), Tyagi II does not disclose any property of such a probe or the probe required by claim 3. Because Tyagi II fails to disclose an amplification target circle, Tyagi II does not disclose any non-complementarity to any amplification target circle. For at least these additional reasons, Tyagi II fails to anticipate claim 3.

For all of the above reasons, claims 1-3, 5-9, 20-28, 30-34 and 59 are not anticipated by Tyagi I.

Rejections Under 35 U.S.C. § 103

1. Claims 1-9, 11-34 and 40-59 were rejected under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 5,925,517 to Tyagi et al. ("Tyagi II"), in view of WO

97/19193 ("Lizardi I"). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

Tyagi II discloses a method of detecting nucleic acid sequences using probes that form stem and loop structures and that produce a detectable signal when the stem is disrupted. Tyagi II does not disclose or suggest altering any of the probes having intact stems and does not disclose altering only the probes having intact stems. Tyagi II also fails to disclose or suggest the use of a rolling circle replication primer as part of a probe.

Claims 1-9, 11-19, 27-34 and 40-59 are drawn to, in part, a method of detecting target nucleic acid sequences comprising

(a) bringing into contact one or more target samples and one or more structured probes, and incubating under conditions that promote hybridization between target nucleic acid sequences in the target samples and the structured probes,

wherein each structured probe has a first end and a second end, wherein each structured probe comprises at least two complementary portions, a target probe portion, and a detection portion, wherein the two or more complementary portions comprise a first complementary portion and a second complementary portion,

wherein two or more of the complementary portions form a duplex region, wherein formation of the duplex region forms a loop, wherein at least a portion of the target probe portion is in the loop, wherein hybridization of the target probe portion to the target nucleic acid sequence disrupts the duplex region,

(b) detecting the structured probes, wherein, prior to detection, the structured probes are treated to alter unhybridized probes, wherein only probes with intact duplex regions are altered by treatment of the structured probes, wherein altered probes are not detected.

Significantly, claim 1 (from which the other claims depend) requires that two or more of the complementary regions of the structured probes form a duplex region together. Claim 1 also requires that this duplex region be disrupted when the structured probe hybridizes to the target nucleic acid sequence. Claim 1 also requires that only probes with intact duplex regions are altered. That is, only structured probes that do not hybridize to the target nucleic acid sequence are altered.

Tyagi II fails to disclose or suggest any alteration of the stem and loop probes used in the Tyagi II method. Tyagi II fails to disclose or suggest alteration of only those stem and loop probes with intact stems. Rather, the detection method of Tyagi II is based only on hybridization versus non-hybridization of probes to a target nucleic acid sequence. There is no alteration of non-hybridized probes either disclosed or required in the method of Tyagi II because hybridization alone determines signal generation in Tyagi II. Thus, Tyagi II fails to disclose or suggest at least these two features of claims 1-3, 5-9, 27, 28, 30-34 and 59: alteration of a structured probe and alteration of only those structured probes with intact duplex regions. Lizardi I was not cited to disclose or suggest these features. Accordingly, Tyagi II and Lizardi I, either alone or in combination, fail to make obvious claims 1-9, 11-34 and 40-59.

Regarding claims 2, 8 and 9, Applicants note that claim 2 (from which the other claims depend) requires that the treatment of the structured probe is extension of the second end of the probe using probe sequence as template and that only probes with intact duplex regions are extended. Tyagi II fails to disclose or suggest either of these features. Tyagi II does not disclose or suggest extension of probes. Rather, Tyagi II discloses (in the passage cited in the Office Action) universal stems (which can have a single-stranded overhang) that can be joined to target sequences to produce a probe for use in the method of Tygai II (col. 19, lines 5-14). Such universal stems are not structured probes and are not extended using probe sequence as template. The amplification (col. 20, lines 40-56) cited in the Office Action refers to general amplification of sample nucleic acids in preparation for probe hybridization. Nothing in the cited passage indicates that the probes are extended in the amplification reactions. Lizardi I was not cited to disclose or suggest these features. Because Tyagi II and Lizardi I fail to disclose or suggest extension of structured probes using probe sequence as template, Tyagi II and Lizardi I, either alone or in combination, fail to make obvious claims 2, 8 and 9 for at least these additional reasons.

Regarding claim 3, Applicants note that claim 3 requires that the extended probe not be complementary to the primer complement of an amplification target circle. Because Tyagi II fails to disclose or suggest the extension required in claim 2 (from which claim 3 depends), Tyagi II does not disclose or suggest any property of such a probe or the probe required by claim

3. Lizardi I was not cited to disclose or suggest this feature. For at least this additional reason, Tyagi II and Lizardi I, either alone or in combination, fail to make obvious claim 3.

Regarding claims 40-43, neither Tyagi II nor Lizardi I specifically disclose or suggest using molecular beacon probes as rolling circle replication primers. For example, Lizardi I discloses the use of molecular beacon probes to detect the products of rolling circle amplification. Tyagi II discloses the use of molecular beacon probes as probes for labeling and detecting target nucleic acid sequences, not as primers. Lizardi I uses molecular beacon probes for the same detection purpose as Tyagi II and neither Lizardi I nor Tyagi II disclose or suggest modifying molecular beacon probes to include a rolling circle replication primer. The separate and distinct use of rolling circle replication primers and molecular beacon probes in Lizardi I suggests only their separate use. For at least these additional reasons, Tyagi II and Lizardi I, either alone or in combination, fail to make obvious claims 40-43.

Regarding claim 4, Lizardi I discloses the use of chain-terminating nucleotides as part of a method of sequencing nucleic acids (see page 93). The chain-terminating nucleotides are labeled such that the terminated primers can be detected and the added nucleotide identified via the label. Contrary to this, the extension of the probe in claim 4 is an alteration of probes that have not hybridized to target nucleic acid sequences and are not to be detected. The extension in claim 4 alters the unhybridized probes so they will not be detected (see step (b) of claim 1). Thus, the principles and operation of the use of chain-terminating nucleotides in Lizardi I is contrary to use of chain-terminating nucleotides in claim 4. Accordingly, the use and method of Lizardi I would not suggest modification of the method of Tyagi II to arrive at the method of claim 4. For at least these additional reasons, Tyagi II and Lizardi I, either alone or in combination, fail to make obvious claim 4.

Regarding claims 11-14, 19 and 42, Applicants note that claim 11 requires that only those probes with intact duplex regions be cleaved. The probes with intact duplex regions are those probes that do not hybridize to the target nucleic acid sequence (see claim 1). Thus, claim 11 requires that unhybridized probes be cleaved and that hybridized probes not be cleaved. In contrast, Lizardi I discloses exonuclease digestion of probes (left over from an earlier step) that hybridize to the products of the amplification method of Lizardi I (see page 73, lines 9-20).

Thus, the digestion of Lizardi I is directed to probes that hybridize, not to probes that are unhybridized and remain intact. Further, Lizardi I fails to disclose or suggest cleavage of only probes with intact duplex regions. The open circle probes discussed on page 73 of Lizardi I are not disclosed to form duplex regions and so there can be no suggestion that such non-existent structures could be the basis for cleavage. For at least these reasons, Lizardi I, either alone or in combination with Tyagi II, fails to disclose or suggest the subject matter of claims 11-14, 19 and 42. For at least these additional reasons, Tyagi II and Lizardi I, either alone or in combination, fail to make obvious claims 11-14, 19 and 42.

Regarding claim 29, Applicants note that claim 29 requires that hybridization of an amplification target circle to a portion of the detection portion of a structured probe not disrupt the duplex region of the probe. That is, the exposed portion of the detection portion to which the amplification target circle could bind is not of sufficient length or composition to allow the duplex region of the structured probe to be disrupted. This has nothing to do with disruption of an amplification reaction, which is what the cited section of Lizardi I concerns. There is no nexus between this disclosure in Lizardi I and the feature recited in claim 29. For at least these additional reasons, Tyagi II and Lizardi I, either alone or in combination, fail to make obvious claim 29.

For all of the above reasons, claims 1-9, 11-34 and 40-59 are not obvious in view of Tyagi II and Lizardi I.

2. Claims 10 and 35-39 were rejected under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 5,925,517 to Tyagi et al. ("Tyagi II"), in view of WO 97/19193 ("Lizardi I") and U.S. Patent No. 6,316,229 to Lizardi ("Lizardi II"). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

Tyagi II discloses a method of detecting nucleic acid sequences using probes that form stem and loop structures and that produce a detectable signal when the stem is disrupted. Tyagi II does not disclose or suggest altering any of the probes having intact stems and does not disclose altering only the probes having intact stems. Tyagi II also fails to disclose or suggest the use of a rolling circle replication primer as part of a probe.

Claims 10 and 35-39 are drawn to, in part, a method of detecting target nucleic acid sequences comprising

(a) bringing into contact one or more target samples and one or more structured probes, and incubating under conditions that promote hybridization between target nucleic acid sequences in the target samples and the structured probes,

wherein each structured probe has a first end and a second end, wherein each structured probe comprises at least two complementary portions, a target probe portion, and a detection portion, wherein the two or more complementary portions comprise a first complementary portion and a second complementary portion,

wherein two or more of the complementary portions form a duplex region, wherein formation of the duplex region forms a loop, wherein at least a portion of the target probe portion is in the loop, wherein hybridization of the target probe portion to the target nucleic acid sequence disrupts the duplex region,

(b) detecting the structured probes, wherein, prior to detection, the structured probes are treated to alter unhybridized probes, wherein only probes with intact duplex regions are altered by treatment of the structured probes, wherein altered probes are not detected.

Significantly, claim 1 (from which the other claims depend) requires that two or more of the complementary regions of the structured probes form a duplex region together. Claim 1 also requires that this duplex region be disrupted when the structured probe hybridizes to the target nucleic acid sequence. Claim 1 also requires that only probes with intact duplex regions are altered. That is, only structured probes that do not hybridize to the target nucleic acid sequence are altered.

Tyagi II fails to disclose or suggest any alteration of the stem and loop probes used in the Tyagi II method. Tyagi II fails to disclose or suggest alteration of only those stem and loop probes with intact stems. Rather, the detection method of Tyagi II is based only on hybridization versus non-hybridization of probes to a target nucleic acid sequence. There is no alteration of non-hybridized probes either disclosed or required in the method of Tyagi II because hybridization alone determines signal generation in Tyagi II. Thus, Tyagi II fails to disclose or suggest at least these two features of claims 10 and 35-39: alteration of a structured probe and

alteration of only those structured probes with intact duplex regions. Lizardi II was not cited to disclose or suggest these features. Accordingly, Tyagi II and Lizardi II, either alone or in combination, fail to make obvious claims 10 and 35-39.

Regarding claim 10, Lizardi II discloses the use of chain-terminating nucleotides as part of a method of sequencing nucleic acids (see cols. 66-67). The chain-terminating nucleotides are labeled such that the terminated primers can be detected and the added nucleotide identified via the label. Contrary to this, the extension of the probe in claim 10 is an alteration of probes that have not hybridized to target nucleic acid sequences and are not to be detected. The extension in claim 10 alters the unhybridized probes so they will not be detected. Thus, the principles and operation of the use of chain-terminating nucleotides in Lizardi II is contrary to use of chain-terminating nucleotides in claim 10. Accordingly, the use and method of Lizardi II would not suggest modification of the method of Tyagi II to arrive at the method of claim 10. For at least these additional reasons, Tyagi II and Lizardi II, either alone or in combination, fail to make obvious claim 10.

Regarding claims 35-39, Applicants note that claims 35-39 involve use of a structured probe that forms a curl stem and loop structure (see claim 34 and Figure 3B). The use of two 3' ends in the claims arises from the need to have anti-parallel complementary portions in a curl stem and loop form of structured probe (see page 10, first paragraph, of the application). Neither Tyagi II nor Lizardi II disclose or suggest forming a probe having a curl stem and loop structure. The probe/primers of Lizardi II that have two 3' ends are not disclosed to form any intramolecular structure. The molecular beacon probes of Tyagi II and Lizardi II are disclosed to form stem and loop structures (see, for example, Fig. 3 in Tyagi II), not curl stem and loop structures. Thus, neither Lizardi II nor Tyagi II disclose or suggest use of probes having the claimed curl stem and loop structure. For at least these additional reasons, Tyagi II and Lizardi II, either alone or in combination, fail to make obvious claim 35-39.

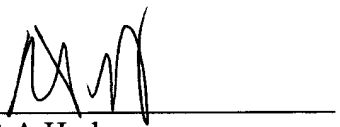
For all of the above reasons, claims 10 and 35-39 are not obvious in view of Tyagi II and Lizardi II.

Pursuant to the above amendments and remarks, reconsideration and allowance of the pending application is believed to be warranted. The Examiner is invited and encouraged to directly contact the undersigned if such contact may enhance the efficient prosecution of this application to issue.

A Credit Card Payment Form PTO-2038 authorizing payment in the amount of $475.00, representing the fee for a small entity under 37 C.F.R. § 1.17(a)(3), and a Request For Extension of Time are enclosed. This amount is believed to be correct; however, the Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-0629.

Respectfully submitted,

NEEDLE & ROSENBERG, P.C.



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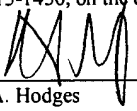

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